Formyltetrahydrofolate synthetase gene diversity in the guts of higher termites with different diets and lifestyles

Elizabeth A Ottesen and Jared R Leadbetter

Supplemental Materials

Supplemental Figure 1. Mitochondrial cytochrome oxidase II phylogeny of termites and roaches.

Supplemental Figure 2. Phylogenetic analysis of termite and roach FTHFS sequences.

Supplemental Figure 3. UniFrac Analysis of FTHFS library compositions.

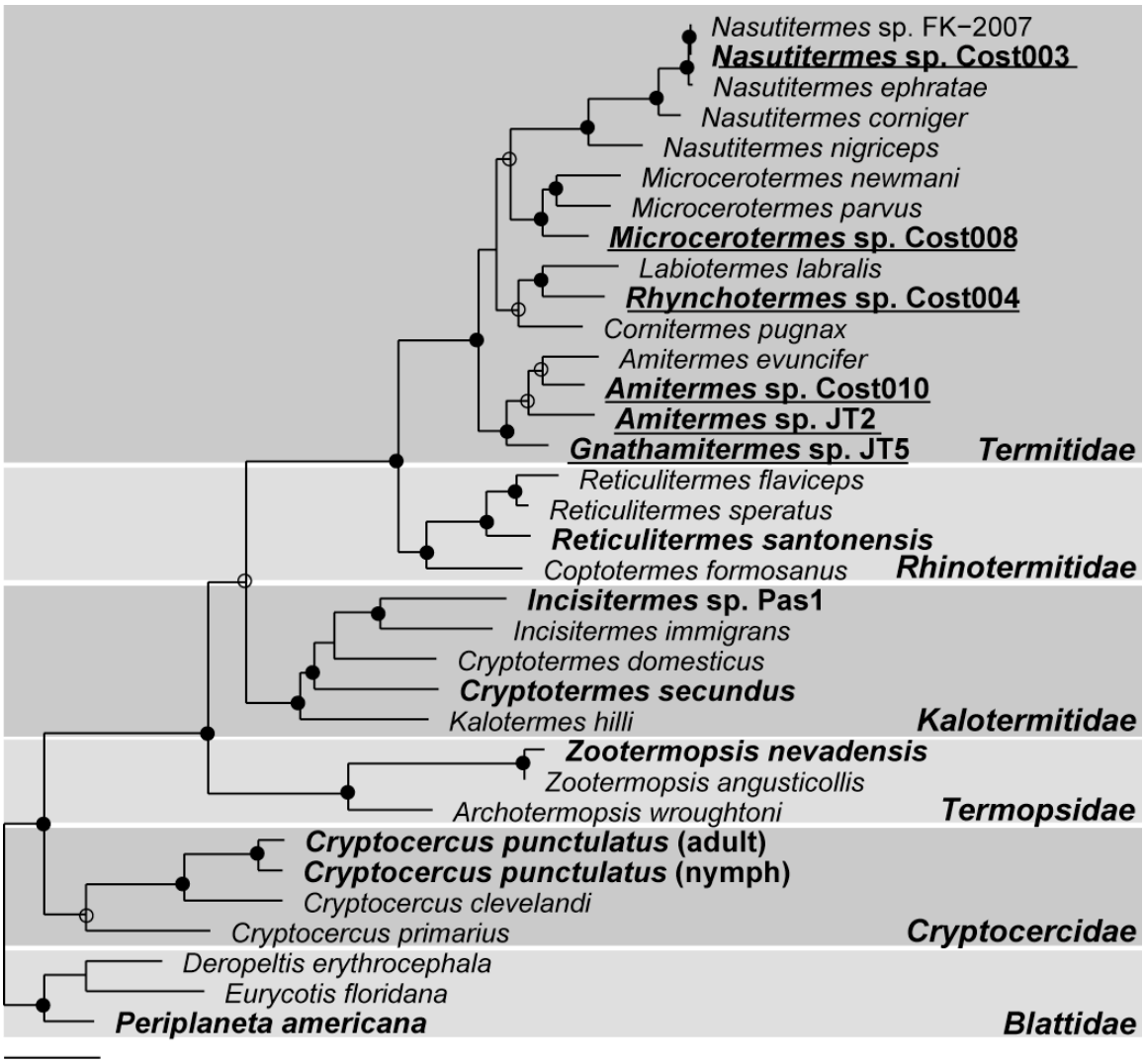
Supplemental Figure 4. Phylogenetic analysis of FTHFS sequences used for UniFrac analysis.

Supplemental Table 1. Operational taxonomic unit grouping of FTHFS sequences identified in this study

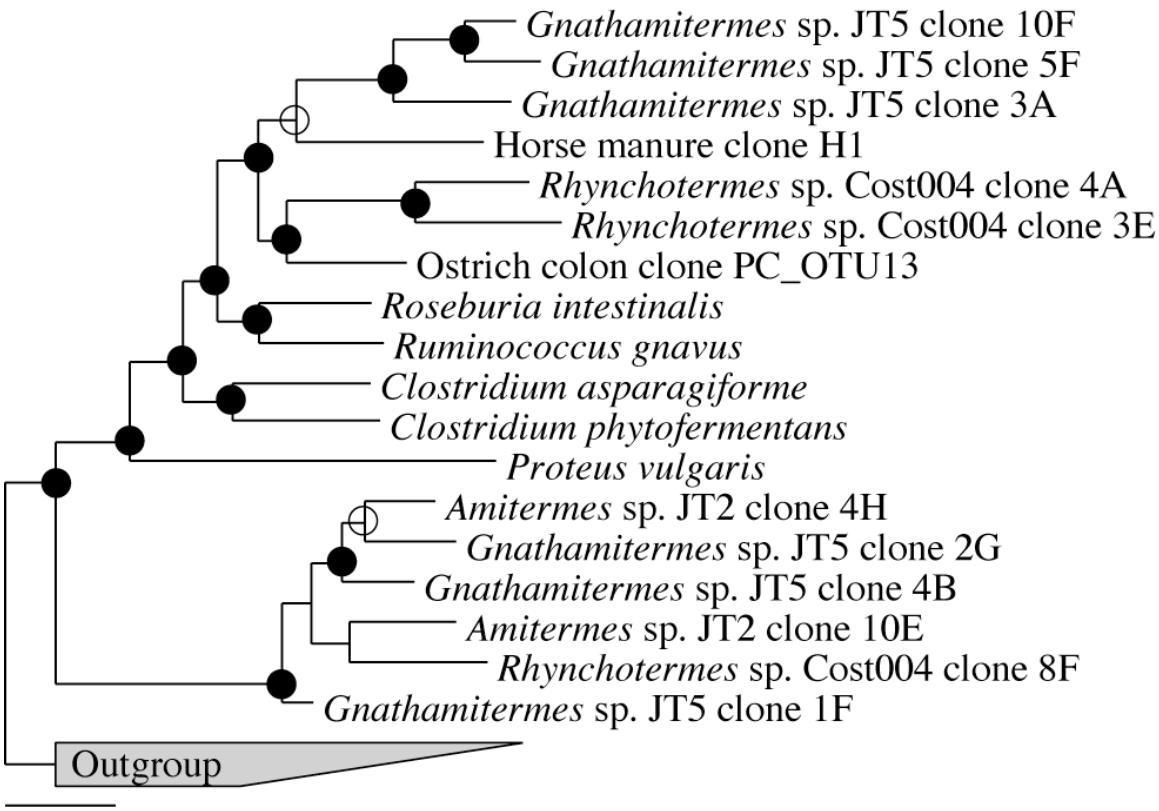
Supplemental Table 2. Sequences used in FTHFS phylogenetic analysis

Supplemental Table 3. Sequences used in COII phylogenetic analysis

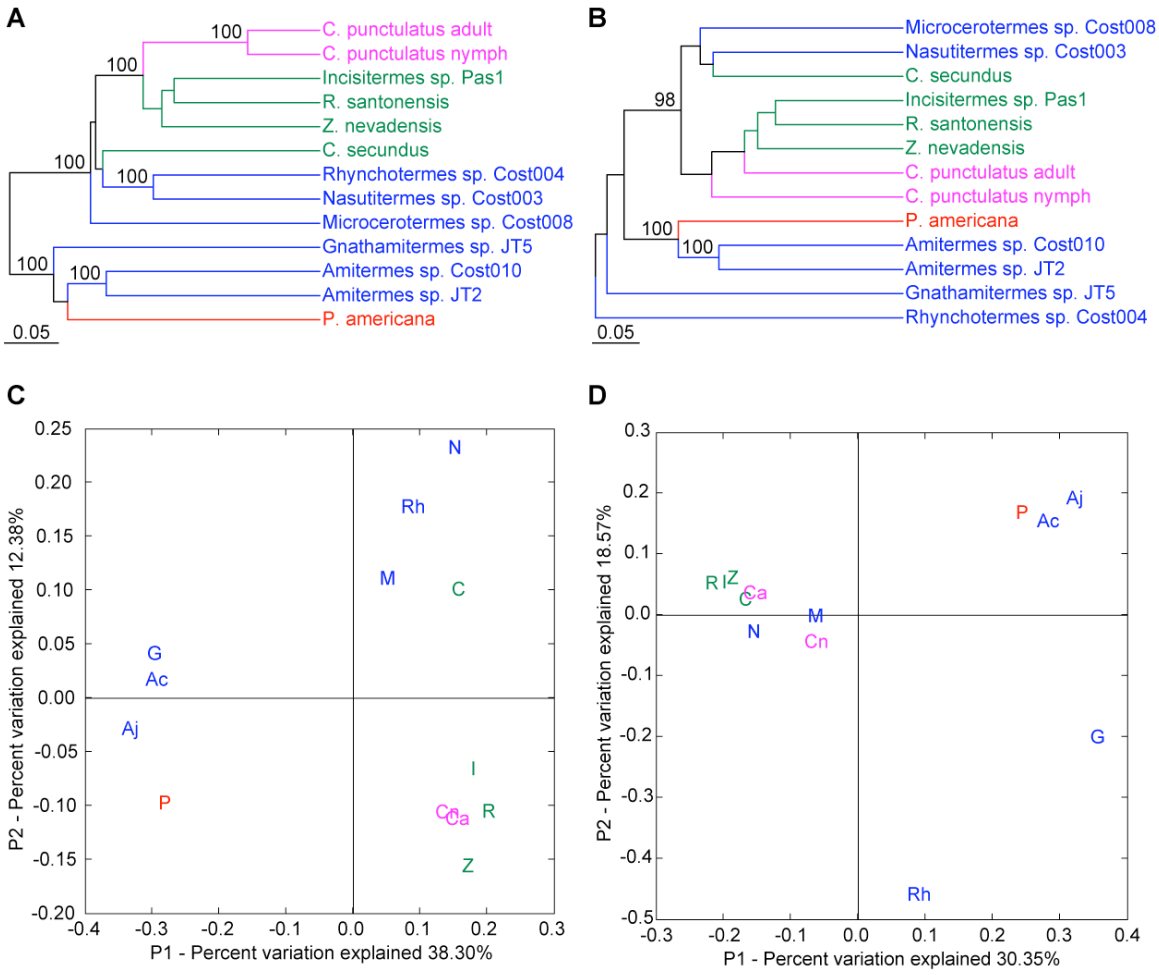
Supplemental References.



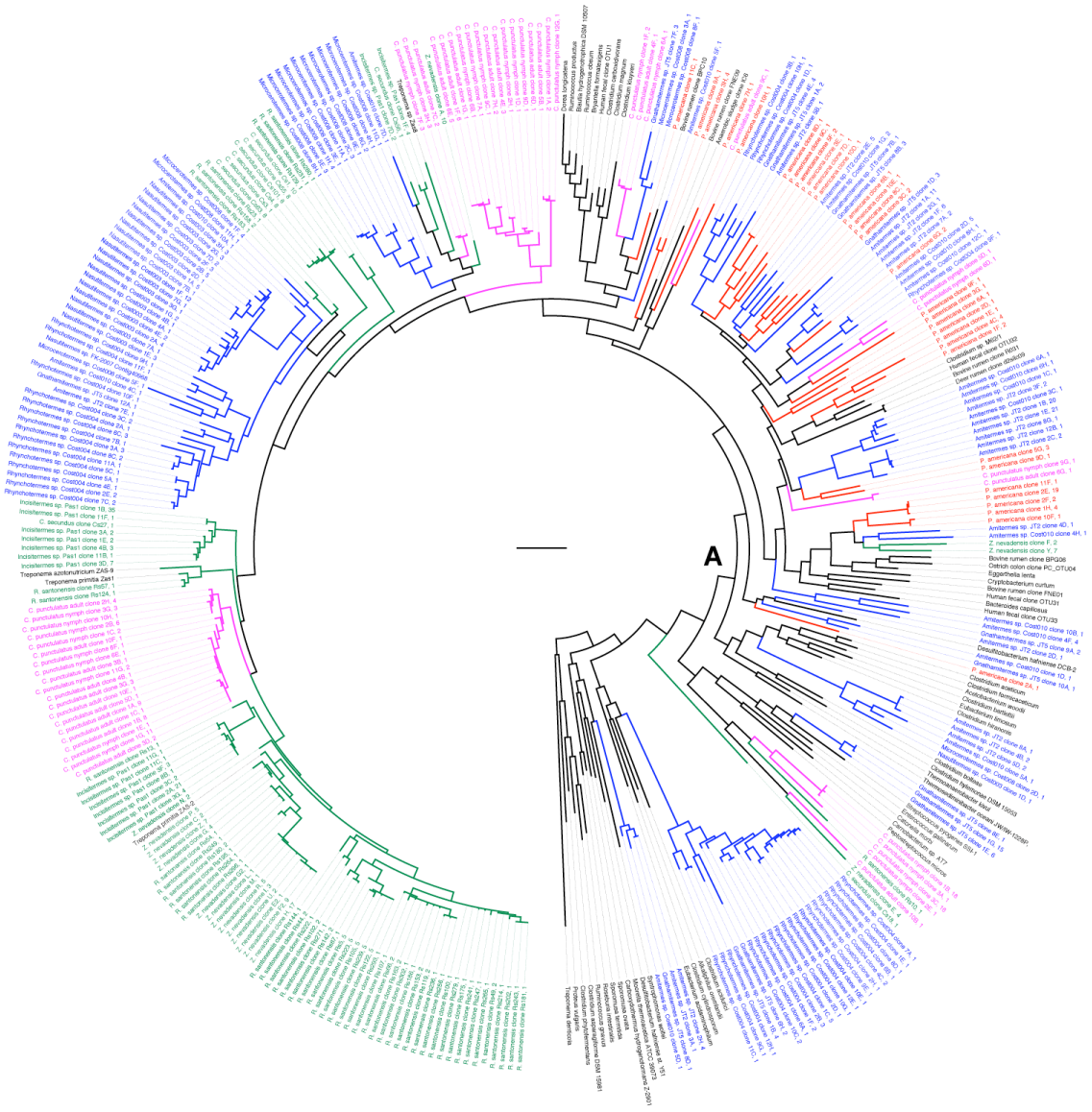
Supplemental Figure 1. Mitochondrial cytochrome oxidase II phylogeny of termites and roaches. Species from which gut FTHFS diversity has been examined marked in bold, species examined in this study underlined. Tree calculated using Phylip PHYML and 393 unambiguous, aligned DNA bases. Open circles indicate nodes also supported by either Fitch distance or Phylip parsimony methods. Closed circles indicate nodes supported by all three algorithms. Scale bar represents 0.1 base pair changes per alignment position.



Supplemental Figure 2. Non-acetogenic Firmicutes. Tree constructed using 346 unambiguous, aligned amino acids and the PhyML maximum likelihood algorithm. Open circles indicate nodes also supported by either Fitch distance or Phylip parsimony methods. Closed circles indicate nodes supported by all three algorithms. Scale bar indicates 0.1 changes per alignment position. The 13 acetogenic isolates in Figure 1 (main text) were used as an outgroup.



Supplemental Figure 3. UniFrac Analysis of FTHFS library compositions. Analyses conducted utilizing the phylogenetic tree shown in Supplemental Figure 4. As the UniFrac Jacknife re-sampling algorithm does not correct for sequence de-replication (6), sequences representing multiple clones were copied and added back into the tree before analysis. Environments were clustered using the abundance-weighted, normalized metric for the Lovell cluster (A) or full tree (B). Nodes supported by >90% of Jacknife re-sampling tests are marked. Principal component analyses were conducted using the abundance-weighted, normalized metric for the Lovell cluster (A) or the full tree (B). Abbreviations: P, *P. americana*; Ca, *C. punctulatus* adult; Cn, *C. punctulatus* nymph; Z, *Z. nevadensis*; R, *R. santonensis*; C, *C. secundus*; I, *Incisitermes* sp. Pas1; N, *Nasutitermes* sp. Cost003; M, *Microcerotermes* sp. Cost008; Rh, *Rhynchotermes* sp. Cost004; G, *Gnathamitermes* sp. JT5; Ac, *Amitermes* sp. Cost010; Aj, *Amitermes* sp. JT2.



Supplemental Figure 4. Phylogenetic analysis of FTHFS sequences used for UniFrac analysis. Tree constructed using 301 unambiguous, aligned amino acids and the PhyML maximum likelihood algorithm. Scale bar indicates 0.1 changes per alignment position. As the PCR primers used in these studies specifically target acetogen-like FTHFS types, UniFrac analyses were conducted for both the Lovell cluster only (node A) and for the Lovell cluster plus the Clone E, *C. acidiurici* and *M. thermoacetica* groups. Clones affiliated with non-target groups such as *Bacteroidetes*, sulfate-reducing *Proteobacteria*, and most non-acetogenic *Firmicutes* were judged to represent non-specific amplification events and were excluded from the analysis. The number of RFLP types represented by each sequence is listed; for *C. secundus* and *R. santonensis*, abundances were published at the phylotype only, so the total number of hits was distributed amongst the representative clones for each group.

SUPPLEMENTAL TABLE 1. Operational Taxonomic Grouping of FTHFS sequences identified in this study

Group	Phylotype[1]	Abundance (%)[2]	Genotypes[3]
***Nasutitermes* sp. Cost003**			
Termite treponemes	1F	23.1%	1F
	2B	15.4%	2B, 2F
	1A	13.5%	1A, 2D, 7B
	2A	9.6%	2A, 7A
	1E	5.8%	1E
	2G	5.8%	2G
	3H	5.8%	3H
	1G	3.8%	1G
	4A	3.8%	4A, 4B
	4E	3.8%	4E
	7D	3.8%	7D
	3G	1.9%	3G
	7G	1.9%	7G
Other	1D	1.9%	1D
***Microcerotermes* sp. Cost008**			
Termite treponemes	1H	29.6%	1H, 5E, 8H
	1F	14.8%	1F
	2C	14.8%	2C, 11A
	6G	11.1%	6G, 4H
	3E	3.7%	3E
	5F	3.7%	5F
	9E	3.7%	9E
	11G	3.7%	11G
	11H	3.7%	11H
Other	2D	3.7%	2D
	3A	3.7%	3A
	8F	3.7%	8F
***Rhynchotermes* sp. Cost004**			
Termite treponemes	7C	9.8%	7C, 2E, 4E, 5A
	3A	8.2%	3A, 8C
	6C	4.9%	6C
	3C	3.3%	3C
	5C	3.3%	5C, 11A
	2A	1.6%	2A
	7B	1.6%	7B
	9H	1.6%	9H
	10F	1.6%	10F
	11F	1.6%	11F
Clostridium sp. M62/1	1D	1.6%	1D
	3B	1.6%	3B
	9F	1.6%	9F
	10H	1.6%	10H
C. acidiurici	1C	13.1%	1C, 2B
	1E	4.9%	1E, 7A, 8D
	4C	4.9%	4C, 6B
	1F	3.3%	1F
	2H	3.3%	2H, 9E
	9G	3.3%	9G, 12H
	10C	3.3%	10C
	10E	3.3%	10E, 12E

Group	Phylotype[1]	Abundance (%)[2]	Genotypes[3]
	2D	1.6%	2D
	6A	1.6%	6A
	10G	1.6%	10G
	11C	1.6%	11C
Other	4A	6.6%	4A
	3E	1.6%	3E
	8F	1.6%	8F
***Amitermes* sp. Cost010**			
Termite treponemes	4C	3.8%	4C
	7D	3.8%	7D
	10A	3.8%	10A
Clostridium sp. M62/1	2D	23.1%	2D, 8H
	4F	15.4%	4F
	1G	7.7%	1G
	1C	3.8%	1C
	3C	3.8%	3C
	6A	3.8%	6A
	6H	3.8%	6H
	10B	3.8%	10B
	12C	3.8%	12C
Other	1D	3.8%	1D
	4H	3.8%	4H
	5A	3.8%	5A
	5D	3.8%	5D
	5F	3.8%	5F
***Amitermes* sp. JT2**			
Termite treponemes	7E	1.1%	7E
Clostridium sp. M62/1	1E	48.3%	1E, 1B, 8G, 12B
	1A	20.2%	1A, 2G, 1F
	2E	5.6%	2E
	2C	2.2%	2C
	3F	2.2%	3F
	7A	2.2%	7A
	5B	1.1%	5B
Other	2H	5.6%	2H, 3A
	5D	5.6%	5D, 8A, 4B
	2D	1.1%	2D
	4D	1.1%	4D
	4H	2.2%	4H, 3D
	10E	1.1%	10E
***Gnathamitermes* sp. JT5**			
Termite treponemes	12A	1.7%	12A
Clostridium sp. M62/1	4E	6.7%	4E
	1D	5.0%	1D
	8B	5.0%	8B
	1A	3.3%	1A
	9A	3.3%	9A
	7B	1.7%	7B
Clone E / *Streptococcus*	1G	26.7%	1G, 8E
	1E	10.0%	1E
C. acidiurici	1B	6.7%	1B
	6H	3.3%	6H
Other	7F	5.0%	7F
	8D	1.7%	8D

Group	Phylotype[1]	Abundance (%)[2]	Genotypes[3]
	10A	1.7%	10A
	3A	6.7%	3A, 9F
	1F	1.7%	1F
	2G	1.7%	2G
	4B	1.7%	4B
	5F	1.7%	5F
	10F	1.7%	10F
Sulfate reducing *Proteobacteria*	2F	1.7%	2F
Not classified	2E	1.7%	2E

[1]Group representative (sequence used in published phylogenetic trees)
[2]Defined as percent of full-length, non-chimeric clones
[3]Sequenced RFLP type clones associated with each group

SUPPLEMENTAL TABLE 2. Sequences used in FTHFS phylogenetic analysis

Source / Sequence Type	Accession	Reference
Acetobacterium woodii	AAK20246	(4)
Alkaliphilus oremlandii	NC_009922	
Blautia hydrogenotrophica DSM 10507	EEG47205	(12)
Bovine rumen clone BPG06	AB085389	
Bovine rumen clone FNE01	AB085446	
Bovine rumen clone R031	AB282722	(8)
Bryantella formatexigens	ZP_05346660	(12)
C. punctulatus adult clone 10B	GU552381	(16)
C. punctulatus adult clone 10F	GU552383	(16)
C. punctulatus adult clone 1A	GU552365	(16)
C. punctulatus adult clone 1F	GU552369	(16)
C. punctulatus adult clone 2H	GU552370	(16)
C. punctulatus adult clone 4F	GU552376	(16)
C. punctulatus adult clone 5B	GU552377	(16)
C. punctulatus adult clone 6D	GU552379	(16)
C. punctulatus adult clone 6G	GU552380	(16)
C. punctulatus adult clone 7C	GU552387	(16)
C. punctulatus adult clone 7H	GU552388	(16)
C. punctulatus nymph clone 1A	GU552335	(16)
C. punctulatus nymph clone 1B	GU552347	(16)
C. punctulatus nymph clone 1F	GU552348	(16)
C. punctulatus nymph clone 1G	GU552337	(16)
C. punctulatus nymph clone 2B	GU552338	(16)
C. punctulatus nymph clone 2E	GU552354	(16)
C. punctulatus nymph clone 2H	GU552355	(16)
C. punctulatus nymph clone 3H	GU552341	(16)
C. punctulatus nymph clone 5D	GU552356	(16)
C. punctulatus nymph clone 6B	GU552343	(16)
C. punctulatus nymph clone 6D	GU552363	(16)
C. punctulatus nymph clone 6F	GU552357	(16)
C. punctulatus nymph clone 7A	GU552358	(16)
C. punctulatus nymph clone 9G	GU552352	(16)
C. secundus clone Cs27	DQ278254	(19)
C. secundus clone Cs3	DQ278251	(19)
C. secundus clone Cs56	DQ278258	(19)
Carboxydothermus hydrogenoformans Z-2901	ABB16038	(29)
Carnobacterium sp. AT7	ABHH01000002	
Catonella morbi	ZP_04449844	(12)
Clostridium aceticum	AF295705	(4)
Clostridium acidurici	P13419	(27)
Clostridium asparagiforme DSM 15981	EEG52107	(12)
Clostridium bartlettii	NZ_ABEZ02000016	(12)
Clostridium bolteae	NZ_ABCC02000077	(12)
Clostridium carboxidivorans	ZP_05391913	(18)
Clostridium cylindrosporum	Q07064	(21)
Clostridium hiranonis	ABWP01000070	(12)
Clostridium hylemonae DSM 15053	EEG73673	(12)
Clostridium phytofermentans	NZ_AAQT01000065	
Clostridium sp. M62/1	ZP_03733593	(12)
Cryptobacterium curtum	YP_003150638	(28)
Deer rumen clone d2silc09	ACZ68327	(1)
Desulfitobacterium hafniense DCB-2	ZP_00559333	
Desulfitobacterium hafniense st. Y51	NC_007907	(13)

Source / Sequence Type	Accession	Reference
Dorea longicatena	NZ_AAXB02000001	(12)
Eggerthella lenta	YP_003183199	(28)
Enterococcus gallinarum	ZP_05648325	(17)
Eubacterium acidaminophilum	AAU84895	
Eubacterium limosum	AF295706	(4)
Horse Manure Clone H1	AF295711	(4)
Human fecal clone OTU32	AB291670	(14)
Incisitermes sp. Pas1 clone 11C	GU552346	(16)
Incisitermes sp. Pas1 clone 1B	GU552321	(16)
Incisitermes sp. Pas1 clone 1E	GU552322	(16)
Incisitermes sp. Pas1 clone 1F	GU552323	(16)
Incisitermes sp. Pas1 clone 2A	GU552324	(16)
Incisitermes sp. Pas1 clone 3A	GU552325	(16)
Incisitermes sp. Pas1 clone 3D	GU552327	(16)
Incisitermes sp. Pas1 clone 7D	GU552331	(16)
Moorella thermoacetica ATCC 39073	ABC18448	(20)
Nasutitermes sp. FK-2007 Contig40968	JGI GOI: 2004144560	(26)
Ostrich colon clone PC_OTU04	BAI48802	(9)
Ostrich colon clone PC_OTU13	BAI48823	(9)
P. americana clone 10D	GU552420	(16)
P. americana clone 10E	GU552421	(16)
P. americana clone 11C	GU552424	(16)
P. americana clone 1E	GU552406	(16)
P. americana clone 1F	GU552390	(16)
P. americana clone 1H	GU552407	(16)
P. americana clone 2D	GU552413	(16)
P. americana clone 2E	GU552394	(16)
P. americana clone 3C	GU552395	(16)
P. americana clone 3E	GU552414	(16)
P. americana clone 3G	GU552415	(16)
P. americana clone 4C	GU552398	(16)
P. americana clone 5F	GU552411	(16)
P. americana clone 5G	GU552399	(16)
P. americana clone 6A	GU552400	(16)
P. americana clone 6B	GU552416	(16)
P. americana clone 6G	GU552401	(16)
P. americana clone 7D	GU552403	(16)
P. americana clone 8C	GU552418	(16)
P. americana clone 8D	GU552429	(16)
P. americana clone 9D	GU552412	(16)
P. americana clone 9F	GU552427	(16)
Peptostreptococcus micros	NZ_ABEE02000017	(12)
Proteus vulgaris	AF295710	(4)
R. santonensis clone Rs10	DQ278259	(19)
R. santonensis clone Rs119	DQ278236	(19)
R. santonensis clone Rs129	DQ278222	(19)
R. santonensis clone Rs13	DQ278232	(19)
R. santonensis clone Rs144	DQ278223	(19)
R. santonensis clone Rs158	DQ278226	(19)
R. santonensis clone Rs23	DQ278210	(19)
R. santonensis clone Rs239	DQ278201	(19)
R. santonensis clone Rs280	DQ278207	(19)
R. santonensis clone Rs296	DQ278208	(19)
R. santonensis clone Rs44	DQ278211	(19)

Source / Sequence Type	Accession	Reference
R. santonensis clone Rs57	DQ278215	(19)
Roseburia intestinalis	NZ_ABYJ01000311	(12)
Ruminococcus gnavus	NZ_AAYG02000005	(12)
Ruminococcus productus	AF295707	(4)
Sporomusa ovata	AF295708	(4)
Sporomusa termitida	AF295709	(4)
Streptococcus dysgalactiae	YP_002997753	
Streptococcus gordonii	NC_009785	(25)
Streptococcus parasanguinis	ZP_06899910	(12)
Streptococcus pyogenes SSI-1	BAC64868	(11)
Streptococcus sanguinis	NC_009009	(30)
Syntrophomonas wolfei	YP_754087	(23)
Thermoanaerobacter kivui	AF295704	(4)
Thermosediminibacter oceani JW/IW-1228P	JGI GOI: 2500808311	
Treponema azotonutricium ZAS-9	AY162316	(22)
Treponema primitia ZAS-1	AY162313	(22)
Treponema primitia ZAS-2	AY162315	(22)
Treponema sp ZAS-8		
Z. nevadensis clone A	AY162294	(22)
Z. nevadensis clone E	AY162296	(22)
Z. nevadensis clone F	AY162298	(22)
Z. nevadensis clone H	AY162302	(22)
Z. nevadensis clone N	AY162306	(22)
Z. nevadensis clone P	AY162307	(22)
Z. nevadensis clone Y	AY162311	(22)

SUPPLEMENTAL TABLE 3. Sequences used in COII phylogenetic analysis

Source	Accession	Reference
Amitermes evuncifer	DQ442066	(3)
Amitermes sp. Cost010	HM208255	
Amitermes sp. JT2	HM208250	
Archotermopsis wroughtoni	DQ442080	(3)
Coptotermes formosanus	AB109529	(15)
Cornitermes pugnax	DQ442106	(3)
Cryptocercus clevelandi	DQ007626	(5)
Cryptocercus primarius	DQ007644	(5)
Cryptocercus punctulatus adult	GU552433	(16)
Cryptocercus punctulatus nymph	GU552434	(16)
Cryptotermes domesticus	AF189086	(24)
Cryptotermes secundus	AF189093	(24)
Deropeltis erythrocephala	DQ874271	(2)
Eurycotis floridana	DQ874283	(2)
Gnathamitermes sp. JT5	HM208249	
Incisitermes immigrans	AB109542	(15)
Incisitermes sp. Pas1	GU552433	(16)
Kalotermes hilli	AF189101	(24)
Labiotermes labralis	DQ442149	(3)
Microcerotermes newmani	DQ442166	(3)
Microcerotermes parvus	DQ442167	(3)
Microcerotermes sp. Cost008	HM208254	
Nasutitermes corniger	AB037327	(10)
Nasutitermes ephratae	AB037328	(10)
Nasutitermes nigriceps	DQ442193	(3)
Nasutitermes sp. FK-2007	EU236539	(26)
Nasutitermes sp. Cost003	HM208252	
Periplaneta americana	GU552435	(16)
Reticulitermes flaviceps	AB109532	(15)
Reticulitermes santonensis	AF291743	(7)
Reticulitermes speratus	AB109530	(15)
Rhynchotermes sp. Cost004	HM208253	
Zootermopsis angusticollis	DQ442267	(3)
Zootermopsis nevadensis	GQ922444	

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